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Estimation and comparison of total carotenoid contents in ethnic vegetables, fruits and some common non leafy vegetables of Bangladesh

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Abstract
18 Leafy (Sabarang, Amila pata, Lalam pata, Baruna shak, Ojan shak, Ghanda batali, Orai balai, bat slai, Maytraba, Dime pata, Maisa pagoh, Sime alu pata, Kongulo aga, Sinei shak, Bat baitta shak, Moikhumu bochuk, Sakum bakla, Taoling asku), 12 Non-Leafy (Sigon data, Fekong, Sengatur/ Thorai, Seon shak, Bash korol, Maira bokong, Laigra bokong, Mistti begun, Tak begun, Pan/Jhum alu, Banchalta, and Bedagi) vegetables and 3 Fruits (Sinera, Roshko, Kushumgulu) from three hilly districts (Bandarban, Rangamati and Khagrachari) of Chittagong Hill Tracts and 7 common non-leafy vegetables (Carrot, Ripe Tomato, Cauliflower, Cabbage, Radish, Bean, Green Tomato) of Bangladesh were estimated for their total carotenoid contents using spectrophotometric method. Among the leafy vegetables, Sime alu pata has the highest and dime pata has the lowest carotenoid content. Among the raw non-leafy vegetables, Agacha baulo has the highest and Fakong has the lowest carotenoid content. Among the 3 fruits from Chittagong Hill Tracts, Sinera has the highest and Kushumgulu has the lowest carotenoid. Among the common non-leafy vegetables, Carrot has the highest and Green Tomato has the lowest carotenoid content and the median value of the total carotenoid content in these common non- leafy vegetables is present in Cabbage. Rich sources for carotenoid among the studied vegetables and fruits are Sime alu pata, Agacha baulo, Maytraba and Carrot in decreasing order respectively.

Keywords: leafy vegetables, non-leafy vegetables, fruits, total carotenoid contents, spectrophotometric method

Introduction

Carotenoids represent one of the most widespread groups of naturally occurring pigments. These compounds are largely responsible for the red, yellow, and orange color of fruits and vegetables, and many dark green vegetables [1]. Carotenoids belong to the category of tetraterpenoids (i.e. they contain 40 carbon atoms). Approximately 50 carotenoids of the known 600 are called "provitamin A" compounds because the body can convert them into retinol, an active form of vitamin A. As a result, foods containing carotenoids can help prevent vitamin A deficiency (VAD). The most commonly consumed provitamin A carotenoids are beta-carotene, alpha-carotene, and beta-cryptoxanthin, but gamma-carotene and beta-zeacarotene also have provitamin A activity [2]. Vitamin A is an essential nutrient which cannot be synthesized by the body and therefore must be provided through diet and needed in small amounts for the normal functioning of the human body [3]. Vitamin A is fat soluble and excessive vitamin A can be stored in the liver, so VAD does not occur immediately when there is no vitamin A in the food, but when the storage in the body has been exhausted [4].

Although micronutrient deficiency or ‘hidden hunger’ such as VAD is a public health problem in Bangladesh, its importance has been underscored through significant investment by national governments and donors to achieve “Millennium Development Goals” relating to mother and child health [5]. In those parts of the world where VAD is prevalent, vegetable products are the main source of dietary vitamin A in the form of carotenoids [6]. Vegetables are the second most important food group after cereals in the diet of Bangladeshi people. In order to carry out nutrition education activities to promote the consumption of carotene-rich products, more accurate data on the composition and content of carotenoids in these foods are required. Considering the above facts, this study is designed;

- To evaluate the total carotenoid contents in ethnic plants growing in the hilly Chittagong districts of Bangladesh and also in some common non-leafy vegetables, and;
- To compare the amount of total carotenoid in the ethnic samples with that of the common non- leafy vegetables.

2. Materials and Methods

2.1. Source and Sampling

33 plants of ethnic origin (18 Leafy, 12 Non-Leafy vegetables and 3 Fruits) and 7 common non-leafy vegetables were estimated for their total carotenoid contents. The ethnic samples were collected from three hilly districts; Bandarban, Rangamati and Khagrachari of Chittagong Hill Tracts in Bangladesh and the common Non-Leafy Vegetables consumed by the mass people of Bangladesh were derived from different local markets and from home gardens. All the samples for the estimation of carotenoid contents were labeled with their local names while collecting.

The available English, local and scientific names of foods included in this study are given in Table 1 and Table 2.

Table 1: List of Ethnic Plants Taken for Estimation of Total Carotenoid Content

Leafy Vegetables			
Serial No.	**English Names**	**Local Names**	**Scientific Names**
1	-	Sabarang	*Ajuga macrosperma*
2	Roselle	Amila pata	*Hibiscus sabdariffa*
3	-	Lalam pata	*Premna obtusifolia*
4	Indian ivy-rue	Baruna shak	*Xanthoxylum rhetsa*
5	-	Ojan shak	*Spilanthes calva*
6	-	Ghanda batali	*Paederia foetida*
7	-	Orai balai	*Premna eaculenta*
8	Purslane	Bat slai	*Portulaca oleracia*
9	Yellow saraca	Maytraba	*Saraca thaipingensis*
10	-	Dime pata	*Glimus oppositifolius*
11	Wild coriander	Maisa pagoh	*Eryngium foetidum*
12	Cassava	Sime alu pata	*Manihot esculenta*
13	-	Kongulo aga	-
14	-	Senai shak	-
15	-	Bat baitta shak	*Commelina benghalensis*
16	-	Moikhumu bachuk	-
17	-	Sakmu bakla	-
18	-	Taoling asku	-
Non-Leafy vegetables			
1	Pea egg plant	Mistti begun	*Solanum spinosa*
2	Solanum	Tak begun	*Solanum virginianum*
3	Sigon data	Sigon data	*Lasia spinosa*
4	Yam	Pan/Jhum alu	*Dioscorea pentaphylla*
5	Fekong	Fakong	*Alpinia nigra*
6	-	Sengatur/Thorai	-
7	-	Betagi	-
8	-	Seon shak	-
9	-	Bash koral	-
10	-	Maira bokong	-
11	-	Laigrao bokong	-
12	-	Agacha Baulo	-
Fruits			
1	Wild melon	Sinera	*Cumis melo*
2	-	Roshko	*Syzygium balsameum*
3	Bead tree	Kushumgulu	*Elaeocarpus angustifolius*

Table 2: List of Common Non-Leafy Vegetables Taken for Total Carotenoid Content Estimation

Common Non-Leafy Vegetables			
Serial No	**English Name**	**Local Name**	**Scientific Name**
1	Carrot	Gajor	*Daucus carota*
2	Tomato(Ripe)	Paka Tomato	*Lycopersicon esculentum*
3	Cauli flower	Phool Kopi	*Brassica oleracea var. botrytis*
4	Cabbage	Badha Kopi	*Brassica oleracea var.capitata*
5	Radish	Mula	*Raphanus sativus*
6	Bean	Shim	*Lablab purpureus*
7	Tomato(Green)	Kacha Tomato	*Lycopersicon esculentum*

2.2. Sample Preparation

All the freshly collected samples were first washed with tap water and then with distilled water. Surface water on the samples was removed using blotting paper to prepare them for analysis. Raw samples were then chopped and mixed manually to reduce the bulk and particle size of the samples that were brought to the laboratory. Only the edible portions of all the vegetables and fruits were selected and inedible portions (i.e., peel, seed, and shell) were removed prior to sample preparation. For ethnic samples edible portion were selected with the help of local peoples. Samples were packed in storage bag, labeled and then preserved under refrigeration. As carotenoids are very much sensitive for oxidation under high temperature, light, acids, all the necessary precautions were taken to avoid losses of carotenoids and to get more accurate results**.**

2.3. Method Followed for Total Carotenoid Estimation

Total carotenoid contents for all the samples under study were estimated by the standard procedures followed in Harvest plus research. Here we used spectro photometric method to estimate total carotenoid contents in samples which is an effective method of quantifying total carotenoid contents.

2.4. Reagents

Acetone (Merck, Germany), Petroleum Ether (BDH, England), 1% BHT (Butylated Hydroxytoluene) solution (0.1g BHT in 100ml acetone), Alumina / Neutral Aluminum Oxide (E. Merck), Anhydrous Sodium Sulfate (Merck, Germany).

2.5. Instruments

Cutting boards, Knives, Spatulas, Marker, Masking tape, cotton; Morters and Pestles; Glasswares such as Glass rods, Conical flasks, Buchner funnels, Measuring cylinders, Separating funnels, Beakers, Volumetric flasks (25ml and 10 ml), Pipettes and Chromatographic columns; Blotting papers, Filter papers, Aluminum foil, Pieces of black cloths; Micro pipettes, Pasteur pipettes, Ependorf tubes; Dryer, Distillation plant, Weighing machine, Spectrophotometer, Nitrogen gas cylinder etc.

2.6. Estimation of Carotenoid from the Sample

2g of each of the samples was mashed in a mortar using a pestle. 25ml of cold acetone and 100µl of 1% BHT were then added to the grinded samples and homogenized carefully for about 3 minutes. The acetone extract was then collected in a conical flask by filtering the homogenate through buchner funnel with filter paper. Acetone was added to the solid residues left on the buchner funnel until the residues become colorless. 20ml of PE (Petroleum Ether) was taken into separating funnel and then acetone extract was added to it. 150ml of distilled water was added by allowing it to flow along the walls of the funnel and then the lower aqueous phase was discarded. Distilled water was added 4-5 times and lower aqueous phase was discarded. Petroleum ether extract was collected through a small funnel with a cotton plug containing anhydrous sodium sulfate into a 25ml volumetric flask. 10 ml of green colored PE extract was taken into chromatographic column for each sample to resolve carotenoid from the extracts. Eluents were collected into 25ml volumetric flasks after chromatographic separation. Chromatography in descending, gravity-flow columns, known as OCC (Open Column Chromatography), is the classical method for separating carotenoids for quantitative analysis. Separation of the carotenoid pigments was followed visually.

Carotenoids in solution obey the Beer–Lambert law, that is, their absorbance is directly proportional to the concentration. Thus, carotenoids were quantified spectro photometrically. In this experiment the absorbance of the eluent was estimated at 450nm. Spectrophotometer was initially set to zero absorbance with the solvent PE (Petroleum Ether), to ensure that the absorbance reading would reflect only the absorbance of the molecules to be quantified i.e carotenoids in plant samples.

The total carotenoid content was calculated using the following formula:

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times \text{volume (mL)} \times 10^4}{A_{1cm}^{1\%} \times \text{sample weight (g)}}$$

Where;

A = absorbance;

Volume = total volume of extract (25 ml);

$A_{1cm}^{1\%}$ = Absorption coefficient of β carotene in Petroleum Ether (2592).

Multiplying by 100 gives the carotenoid content in µg/100 g.

After spectrophotometric quantification, 1ml of the eluents was preserved in the ependorf tube by drying under nitrogen gas and keeping under refrigeration for further carotenoid profiling by HPLC.

Carotenoid analysis is inherently difficult. The main problem in the analysis of carotenoids stems from their instability. Thus, precautionary measures to avoid artifacts and quantitative losses were taken.

3. Results and Discussion

A Standerdized method of Harvestplus research was followed for all the laboratory techniques needed to estimate the carotenoid contents in the samples. The samples were duplicated for each of the procedures and absorbances of the duplicated samples were taken and then the mean absorbance was also calculated. The values of the absorbance were put in the formula stated in the previous chapter and the corresponding values for total carotenoid contents were estimated. Finally, the mean values of the carotenoid contents were derived. Standard deviations for the carotenoid contents were estimated also. The values of carotenoid contents of all the samples were expressed in mg/100g.

The mean values of total carotenoid contents of raw vegetables and fruits are given in Tables 3- 6:

Table 3: Total Carotenoid Content (mg/100g) in Ethnic Leafy Vegetables

Serial no	English Names	Local Names	Scientific Names	Total Carotenoid Contents (mg/100g)
1	-	Sabarang	*Ajuga macrosperma*	5.96 ± 0.15
2	Roselle	Amila pata	*Hibiscus sabdariffa*	4.41 ± 0.08
3	-	Lalam pata	*Premna obtusifolia*	3.03 ± 0.13
4	Indian ivy-rue	Baruna shak	*Xanthoxylum rhetsa*	6.11 ± 0.27
5	-	Ojan shak	*Spilanthes calva*	4.61 ± 0.38
6	-	Ghanda batali	*Paederia foetida*	6.99 ± 0.10
7	-	Orai balai	*Premna eaculenta*	4.45 ± 0.08
8	Purslane	Bat slai	*Portulaca oleracia*	2.24 ± 0.14
9	Yellow saraca	Maytraba	*Saraca thaipingensis*	13.18 ± 1.15
10	-	Dime pata	*Glimus oppositifolius*	0.8 ± 0.11
11	Wild coriander	Maisa pagoh	*Eryngium foetidum*	1.31 ± 0.02
12	Cassava	Sime alu pata	*Manihot esculenta*	19.73 ± 1.61
13	-	Kongulo aga		1.83 ± 0.02
14	-	Senai shak	-	1.85 ± 0.02
15	-	Bat baitta shak	*Commelina benghalensis*	4.18 ± 0.36
16	-	Moikhumu bachuk	-	1.04 ± 0.03
17	-	Sakmu bakla	-	4.07 ± 0.49
18	-	Taoling asku	-	3.85 ± 0.17

Values are mean (mg/100g) of duplicate determinations on fresh weight basis

In the Table 3; the total carotenoid contents of 18 ethnic raw leafy vegetables are depicted. Here the highest carotenoid content is present in Sime alu pata and lowest in Dime pata, which are 19.73 ± 1.61 mg/100g and 0.8 ± 0.11 mg/100g respectively. The next higher value is found in Maytraba which is 13.18 ± 1.15 mg/100g. The next relative high amounts of carotenoids are contained in Ghanda batali, Baruna shak, Sabarang, Ojan shak, Orai balai, Amila pata, Bat baitta shak, Sakmu bakla with the values 6.99 ± 0.10 mg/100g, 6.11 ± 0. 27 mg/100g, 5.96 ± 0.15 mg/100g, 4.61 ± 0.38 mg/100g, 4.45 ± 0.08 mg/100g, 4.41 ± 0.08 mg/100g, 4.18 ± 0.36 mg/100g, 4.07 ± 0.49 mg/100g respectively.

Table 4: Total carotenoid content (mg/100g) in ethnic Non Leafy Vegetables

Serial	English Names	Local Names	Scientific Names	Total Carotenoid Contents (mg/100g)
1	Pea egg plant	Mistti begun	*Solanum spinosa*	3.62 ± 0.18
2	Solanum	Tak begun	*Solanum virginianum*	4.58 ± 0.24
3	Sigon data	Sigon data	*Lasia spinosa*	0.95 ± 0.01
4	Yam	Pan/jhum alu	*Dioscorea pentaphylla*	0.48 ± 0.01
5	Fekong	Fakong	*Alpinia nigra*	0.13 ± 0.01
6	-	Sengatur/thorai	-	1.00 ±.07
7	-	Betagi	-	0.82 ± 0.03
8	-	Seon shak	-	6.77 ± 0.13
9	-	Bash koral	-	0.75 ± 0.03
10	-	Maira bokong	-	3.98 ± 0.18
11	-	Laigrao bokong	-	3.24 ± 0.07
12	-	Agacha baulo	-	15.14 ± 0.13

Values are mean (mg/100g) of duplicate determinations on fresh weight basis

In the Table 4; the total carotenoid contents in 12 ethnic raw non-leafy vegetables are listed. Here the highest carotenoid content is present in Agacha baulo and lowest in Fakong, which are 15.14 ± 0.13 mg/100g and 0.13 ± 0.01 mg/100g respectively. The next higher value is found in Seon shak which is 6.77 ± 0.13 mg/100g. The next relative high amounts of carotenoids are contained in Tak begun, Maira bokong, Mistti begun, Laigrao bokong with the values 4.58 ± 0.24 mg/100g, 3.98 ± 0.18 mg/100g, 3.62 ± 0.18 mg/100g and 3.24 ± 0.07 mg/100g respectively.

Table 5: Total Carotenoid Content (mg/100g) in Ethnic Fruits

Serial	English Names	Local Names	Scientific Names	Total Carotenoid Contents (mg/100g)
1	Wild melon	Sinera	*Cumis melo*	1.84 ± 0.04
2	-	Roshko	*Syzygium balsameum*	1.19 ± 0.04
3	Bead tree	Kushumgulu	*Elaeocarpus angustifolius*	0.26 ± 0.01

Values are mean (mg/100g) of duplicate determinations on fresh weight basis

In Table 5; the total carotenoid contents in three raw ethnic fruits are showed. The highest carotenoid content is present in Sinera and lowest in Kushumgulu, which are 1.84 mg/100g with SD value ± 0.04 and 0.26 mg/100g with SD value ± 0.01, respectively. Roshko have the carotenoid content in between Sinera and Kushumgulu, which is 1.19 mg/100g with SD value ±0.04.

Table 6: Total Carotenoid Content (mg/100g) in Common Non-Leafy Vegetables

Serial	English Names	Local Names	Scientific Names	Total Carotenoid Contents (mg/100g)
1	Carrot	Gajor	*Daucus carota*	10.77 ± 0.18
2	Tomato (Ripe)	Paka Tomato	*Lycopersicon esculentum*	7.26 ± 0.36
3	Cauli flower	Phool Kopi	*Brassica oleracea var. botrytis*	2.68 ± 0.17
4	Cabbage	Badha Kopi	*Brassica oleracea var.capitata*	0.77 ± 0.02
5	Radish	Mula	*Raphanus sativus*	0.52 ± 0.01
6	Bean	Shim	*Lablab purpureus*	0.42 ± 0.06
7	Green Tomato	Kacha Tomato	*Lycopersicon esculentum*	0.37 ± 0.01

Values are mean (mg/100g) of duplicate determinations on fresh weight basis

In Table 3.4; the total carotenoid contents of seven common raw non-leafy vegetables are shown in decreasing order of their value. Here the highest carotenoid content is present in Carrot and lowest in Green Tomato, which are 10.77mg/100g with SD value ± 0.18 and 0.42mg/100g with SD value ±0.06 respectively. The median value of the total carotenoid content in these common non- leafy vegetables is present in 4th sample vegetable which is Cabbage, having carotenoid content of 0.77 mg/100g with SD value ± 0.02.

Table 7: Rich Sources for Carotenoid in Ethnic and Common Vegetables under study

Serial	English Names	Local Names	Scientific Names	Total Carotenoid Contents (mg/100g)
1	Cassava	Sime alu pata	*Manihot esculenta*	19.73 ± 1.61
2	-	Agacha Baulo	-	15.14 ± 0.13
3	Yellow saraca	Maytraba	*Saraca thaipingensis*	13.18 ± 1.15
4	Carrot	Gajor	*Daucus carota*	10.77 ± 0.18

Values are mean (mg/100g) of duplicate determinations on fresh weight basis

The above table depicts the values for carotenoid contents in Sime alu pata, Agacha baulo & Maytraba and Carrot in decreasing order respectively.
By comparing the values of carotenoid in all the samples of our study, we can easily note that, Sime alu pata, Agacha baulo & Maytraba have higher amounts of carotenoids than Carrot, which which is considered as the richest of carotenoid foods and give this nutrient group its name which was first coined in the early 19th Century by the scientist Wachenroder after he crystallized this compound from carrot roots [7]. So, we can draw an inference that, our neglected and unnoticed indigenous plants can also fulfill our nutrition demand.
The current study of some ethnic plant variety and other common vegetables may help to some extent to develop a national food composition database, which is especially essential for clinical sector.

4. Conclusion

- UN in the year 2000 provides an opportunity for concerted action to improve global health by declaring "Millennium Development Goal (MDG)". In this development program first, fourth, fifth, sixth and eighth goals are; eradicating extreme poverty and hunger, reducing child mortality, improving maternal health, combating HIV/AIDS, malaria and other diseases; and develop a global partnership for development respectively. Park [8] reported that, to achieve these goals, overcoming micronutrient deficiency especially VAD is compulsory. Our current research may contribute in some way to achieve these goals by, Informing indigenous and common people about the rich nutritional content of these plants which are cheap alternatives of animal sources of vitamin- A
- Helping to construct a food chart.
- Conducting nutritional education or communication to improve practices related to the consumption of available vitamin A to combat Vitamin A deficiency.
- Lowering pressure on common food sources of vitamins and minerals and to achieve food security.
- To serve the malnourished people of our country and abroad.
- Encouraging researchers for further investigations on these plants for their effect on human health.

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